Non-tuberculous Mycobacteria isolated from lymph nodes and faecal samples of healthy slaughtered cattle and the abattoir environment

Ghielmetti, G ; Friedel, Ute ; Scherrer, S ; Sarno, E ; Landolt, P ; Dietz, Olivier ; Hilbe, Monika ; Zweifel, Claudio ; Stephan, Roger

Abstract: Infections caused by non-tuberculous mycobacteria (NTM) are reported as emerging disease in many countries worldwide. The occurrence of NTM in different hosts and their implication as obligate or opportunistic pathogen remain largely unclear. Lymph nodes and faecal samples of clinically healthy Swiss cattle at slaughter were analysed for the presence of NTM. Based on the examined lymph nodes, NTM were detected in 20% of 108 cattle originating from different premises. The 22 isolates belonged to five different species of Mycobacteria (M. avium subsp. hominissuis, M. kansasii, M. persicum, "M. lymphaticum" and M. europaeum). M. avium subsp. hominissuis (63%) and M. kansasii (18%) thereby predominated and were found in lymph nodes with and without macroscopic changes. Moreover, M. persicum found in two cattle has recently been described as a human pathogen and is closely related to M. kansasii. Amongst cattle with lymph nodes positive for mycobacteria, viable NTM were occasionally also detected in bovine faeces. However, the isolated NTM species from lymph nodes and respective faecal samples (M. hassiacum, M. phlei and M. vaccae) did not coincide. Moreover, NTM species identified amongst isolates from the slaughterhouse environment clearly differed from those from lymph nodes and faecal samples, excluding cross-contamination of the tissue specimens through the environment or laboratory processing. Assuming that some NTM interfere with the detection of bovine tuberculosis (bTB), the present findings in healthy animals emphasize the need of more specific diagnostic tools for bTB eradication programs.

DOI: https://doi.org/10.1111/tbed.12793

DOI: https://doi.org/10.1111/tbed.12793

Received: 29 August 2017

DOI: 10.1111/tbed.12793

ORIGINAL ARTICLE

WILEY Transboundary and Emerging Diseases

Non-tuberculous *Mycobacteria* isolated from lymph nodes and faecal samples of healthy slaughtered cattle and the abattoir environment

G. Ghielmetti[1] | U. Friedel[1] | S. Scherrer[1] | E. Sarno[2] | P. Landolt[1] | O. Dietz[1] | M. Hilbe[3] | C. Zweifel[2] | R. Stephan[2]

[1]Institute of Veterinary Bacteriology, Swiss Reference Laboratory for bovine tuberculosis, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

[2]Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

[3]Institute of Veterinary Pathology, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

Correspondence
Giovanni Ghielmetti, Institute of Veterinary Bacteriology, Vetsuisse Faculty University of Zurich, Zurich, Switzerland.
Email: giovanni.ghielmetti@vetbakt.uzh.ch

Summary

Infections caused by non-tuberculous mycobacteria (NTM) are reported as emerging disease in many countries worldwide. The occurrence of NTM in different hosts and their implication as obligate or opportunistic pathogen remain largely unclear. Lymph nodes and faecal samples of clinically healthy Swiss cattle at slaughter were analysed for the presence of NTM. Based on the examined lymph nodes, NTM were detected in 20% of 108 cattle originating from different premises. The 22 isolates belonged to five different species of *Mycobacteria* (*M. avium* subsp. *hominissuis*, *M. kansasii*, *M. persicum*, "*M. lymphaticum*" and *M. europaeum*). *M. avium* subsp. *hominissuis* (63%) and *M. kansasii* (18%) thereby predominated and were found in lymph nodes with and without macroscopic changes. Moreover, *M. persicum* found in two cattle has recently been described as a human pathogen and is closely related to *M. kansasii*. Amongst cattle with lymph nodes positive for mycobacteria, viable NTM were occasionally also detected in bovine faeces. However, the isolated NTM species from lymph nodes and respective faecal samples (*M. hassiacum*, *M. phlei* and *M. vaccae*) did not coincide. Moreover, NTM species identified amongst isolates from the slaughterhouse environment clearly differed from those from lymph nodes and faecal samples, excluding cross-contamination of the tissue specimens through the environment or laboratory processing. Assuming that some NTM interfere with the detection of bovine tuberculosis (bTB), the present findings in healthy animals emphasize the need of more specific diagnostic tools for bTB eradication programs.

KEYWORDS

abattoir, cattle, *Mycobacterium avium*, *Mycobacterium kansasii*, *Mycobacterium persicum*, non-tuberculous mycobacteria

1 | INTRODUCTION

The genus *Mycobacterium* comprises nowadays over 180 species, ranging from harmless saprophytes to significant pathogens. Besides the members of the well-known *Mycobacterium tuberculosis* complex (MTBC), for example, *M. bovis* or *M. tuberculosis*, a great variety of non-tuberculous mycobacteria (NTM) have been described (Procop, 2017; Tortoli, 2003). NTM are commonly encountered in the

environment and they have been isolated from a variety of sources, including water, feed, soil, dust, aerosol, invertebrates, protozoa or animals (Biet & Boschiroli, 2014; Falkinham, 2015). Some NTM are opportunistic pathogens, which might be transmitted between the environment, wildlife, livestock and humans (Biet, Boschiroli, Thorel, & Guilloteau, 2005; Falkinham, 2009). More than 60 species of NTM are known to be pathogenic to humans and animals (Biet & Boschiroli, 2014; Falkinham, 1996; Griffith et al., 2007; Tortoli, 2003). A large variety of clinical manifestations caused by NTM are described, amongst others lymphadenitis, lung disease, skin infections, soft tissue infections and visceral or disseminated disease (Falkinham, 1996; Griffith et al., 2007). In humans, NTM-associated infections in immunocompetent persons have recently increased (Prevots & Marras, 2015; Primm, Lucero, & Falkinham, 2004). Identification of mycobacteria at species level is therefore crucial for evaluation of their clinical significance.

Despite the increasing interest in NTM infections, information on the occurrence and the diversity of NTM in livestock, especially in cattle, is still restricted. The available literature is mainly focused on (i) the *Mycobacterium avium* complex (MAC) and its subspecies, (ii) NTM derived from clinical samples suspicious for MTBC and (iii) isolates from pigs (Agdestein, Olsen, Jorgensen, Djonne, & Johansen, 2014; Klanicova-Zalewska & Slana, 2014; Lara et al., 2011; Leao et al., 2014; Muwonge et al., 2014; Vluggen et al., 2016). Only a few recent studies (France, Hungary, South Africa, South Korea, Tanzania, US) comprehensively evaluated the spectrum of NTM species in cattle using molecular methods (Biet & Boschiroli, 2014; Gcebe, Rutten, Gey van Pittius, & Michel, 2013; Katale et al., 2014; Kim et al., 2014; Ronai et al., 2016; Thacker, Robbe-Austerman, Harris, Van Palmer, & Waters, 2013). Another aspect of veterinary relevance deserving attention is the interference of certain NTM species, for example *M. kansasii* and members of the MAC, with the diagnosis of bovine tuberculosis (bTB) (Biet & Boschiroli, 2014; de la Rua-Domenech et al., 2006; Hope et al., 2005; Schiller et al., 2010; Thacker et al., 2013; Vordermeier et al., 2007). Ante-mortem diagnosis of bTB is commonly based on tuberculin skin tests and interferon gamma (IFN-γ) release assays. Non-specific sensitization (cross-reactions) due to the exposure or infection with NTM species displaying the same antigen(s) as the target species therefore represents a serious issue for bTB eradication. Thus, the aim of this study was (i) to determine the occurrence and to identify the species of mycobacteria prevalent amongst healthy slaughtered cattle in Switzerland, and (ii) to compare the species distribution of mycobacteria from lymph nodes, faecal samples and the slaughterhouse environment.

2 | MATERIALS AND METHODS

2.1 | Abattoir and sample collection

This study was based on investigations carried out in a Swiss abattoir with an annual slaughter capacity of more than 20 million kg (cattle, sheep and pigs). The abattoir processed up to 60 cattle carcasses per hour (on average 85 cattle carcasses per day). During 2 months (January to February 2015), a total of 108 healthy cattle were sampled. Sampled cattle aged between 3 and 24 months, and they originated from the north and central part of Switzerland. From animals delivered together to the abattoir and originating from the same premise, not more than one animal was included in the survey. Sampling comprised five sampling days and between 11 and 35 cattle per sampling day.

From each of the 108 sampled cattle, two lymph nodes (left bronchial lymph node and caudal mediastinal lymph node) and a faecal sample were collected. Lymph nodes were excised after evisceration using sterile forceps and scissors. Faecal samples were collected after evisceration from the large intestine using swabs. In addition, 15 environmental samples were collected during the slaughtering process on three occasions using swabs moistened with 0.85% saline solution. Environmental samples were obtained from the slaughter line ($n = 3$), hooks for the thoracic viscera and the liver ($n = 4$), backside and palm side of gloves ($n = 3$), blade of the knife that the butcher used to cut respiratory tract lymph nodes ($n = 2$), the wall behind the suspended respiratory tract ($n = 2$) and a water handle ($n = 1$). Lymph nodes, faecal samples and environmental swabs were packed into sterile stomacher bags and transported to the laboratory chilled.

2.2 | Sample preparation

Lymph nodes were trimmed of fat and connective tissue upon arrival in the laboratory. Faecal samples were temporarily stored at −20°C. Only faecal samples from cattle with lymph nodes positive for *Mycobacterium* spp. were investigated. The microbiological analysis of the samples was performed according to Ghielmetti et al. (Ghielmetti et al., 2017). Briefly, 2 g of each sample was homogenized in 20 ml saline solution (0.9%) using a rotating-blade macerator system (T18 Digital Ultra-Turrax IKA, Staufen, Germany) and subsequently centrifuged 15 min at 3000 *g*. After discarding the supernatant, the sediment was decontaminated by suspension in 4.0 ml H_2SO_4 (4%), incubation for 15 min at ambient temperature and neutralization by adding 5.65 ml NaOH (1 M). Afterwards, 20 ml phosphate-buffered saline solution (PBS, pH 7.4) was added and the suspension centrifuged 15 min at 3000 *g*. The final sediment was resuspended in 2.5 ml PBS and used as inoculum. Swabs originating from the environmental sampling were washed into 4 ml saline solution (0.9%) and subsequently processed in the same manner as specimen of animal origin.

2.3 | Mycobacterial culture

Two BBL MGIT liquid media tubes supplemented with Bactec MGIT 960 growth supplement, BBL MGIT PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) antibiotic mixture (Becton, Dickinson, BD, Allschwil, Switzerland) and 50 μg/ml sodium pyruvate were each inoculated with 0.5 ml of the inoculum and incubated up to 8 weeks at 37°C in a BACTEC MGIT 320 incubator (BD). Cultures were checked regularly for growth. In order to obtain pure mycobacterial cultures, subcultures on 7H10 agar slants (BD) and on BBL Stonebrink agar slants (BD) were performed at intervals of 3–10 days. Cultures showing growth of presumptive

mycobacterial colonies were checked for acid-fast bacilli using Ziehl–Neelsen staining.

2.4 | DNA extraction

DNA from cultured mycobacteria was extracted through mechanical cell lysis using a TissueLyser II (Qiagen, Hilden, Germany) and enzymatic digestion with Proteinase K (Qiagen) overnight. Automated DNA purification was performed using the QIAcube instrument in accordance with the QIAamp cador Pathogen Mini Kit protocol (Qiagen). DNA concentration in the final eluate was measured by reading the absorbance at 260 nm using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Reinach, Switzerland), diluted to a concentration of 10 ng/μl and stored at −20°C until use.

2.5 | DNA amplification and molecular testing

Purified DNA was used for direct MTBC detection by *artus® M. tuberculosis* PCR Kit (Qiagen) and the 7500 Fast real-time PCR system (7500 Fast; Applied Biosystems, Zug, Switzerland). Pure cultures that presented acid-fast bacilli by Ziehl–Neelsen staining and negative MTBC PCR results were classified as NTM and included in the study. NTM were further identified by PCR amplification and sequencing of the 16S rRNA (Kirschner & Böttger, 2000), combined with sequencing of the Adékambi region of the *rpoB* gene (Adékambi, Colson, & Drancourt, 2003). Additionally, a 441-bp portion of the *hsp65*-encoding gene was sequenced for each cultured mycobacterial species with exception of the MAC isolates (Telenti et al., 1993). For the latter, the complete *hsp65* gene was sequenced as proposed by Turenne et al. (Turenne, Semret, Cousins, Collins, & Behr, 2006). DNA sequencing was performed at Microsynth (Balgach, Switzerland). Resulting sequences were assembled using CLC Genomics Workbench 7.5.1 (Qiagen) and BLAST similarity searching for multiple sequence alignment was performed (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Control strains included were *M. avium* subsp. *avium* ATCC 25291, *M. avium* subsp. *hominissuis* ATCC 700898, *M. peregrinum* ATCC 700686, *M. kansasii* ATCC 12478 and *M. gastri* DSM 43505. The variable SNPs alignment of the cultured *M. kansasii* complex isolates was used to infer a maximum likelihood phylogeny using the neighbour-joining method (Saitou & Nei, 1987) bootstrapped 1000 times, supported by the CLC Genomics Workbench 7.5.1 (Qiagen).

2.6 | Macroscopic and histological examination

After trimming lymph nodes of fat and connective tissue, qualified staff inspected them macroscopically and recorded potential pathological changes. A subset of lymph nodes was additionally sampled for histology. The focus was thereby on lymph nodes from cattle testing positive for mycobacteria and presenting macroscopic lesions. Samples for histological examination were fixed in 10% buffered formalin and embedded in paraffin. Two- to three-micron-thick tissue sections were obtained and stained with haematoxylin and eosin.

2.7 | Ethics statement

Animals used in this study did not undergo any manipulation prior to stunning for standard industrial slaughter according to the pertinent legislation. For this reason, no specific ethical approval was required.

3 | RESULTS

All mycobacterial isolates from lymph nodes, faecal samples and environmental did not belong to the MTBC and tested negative by *artus® M. tuberculosis* real-time PCR.

3.1 | Lymph nodes and faecal samples

Of the 108 sampled healthy cattle at slaughter, lymph nodes from 22 (20%) cattle showed typical growth of *Mycobacterium* spp. and detection of acid-fast bacilli by Ziehl–Neelsen staining in the mycobacterial cultures. The sequenced genes (16S rRNA, *rpoB*, *hsp65*) assigned the 22 isolates to five different mycobacterial species, namely *M. avium* subsp. *hominissuis*, *M. kansasii*, *M. persicum*, "*M. lymphaticum*" and *M. europaeum* (Table 1). *M. avium* subsp. *hominissuis* thereby predominated with 63% of the isolates, followed by *M. kansasii* with 18% of the isolates. "*M. lymphaticum*" is a novel

TABLE 1 *Mycobacterium* spp. isolated from slaughtered Swiss cattle and the slaughterhouse environment from January to February 2015

	***Mycobacterium* spp. from cattle and the abattoir environment**	
	Species identified	**No. of isolates**
Lymph nodes[a] (22 NTM isolates)	*Mycobacterium avium* subsp. *hominissuis*	14
	Mycobacterium kansasii	4
	Mycobacterium persicum	2
	"Mycobacterium lymphaticum"	1
	Mycobacterium europaeum	1
Faecal samples[b] (three NTM isolates)	*Mycobacterium hassiacum*	1
	Mycobacterium phlei	1
	Mycobacterium vaccae	1
Environment[c] (nine NTM isolates)	*Mycobacterium paragordonae*	6
	Mycobacterium peregrinum	2
	Mycobacterium nebraskense	1

[a]Lymph nodes (left bronchial lymph node and caudal mediastinal lymph node) from 108 cattle were examined for *Mycobacterium* spp.
[b]Only faecal samples from the 22 cattle with positive lymph nodes were examined for *Mycobacterium* spp.. Besides the three *Mycobacterium* spp. isolates, one *Rhodococcus equi* and two *Nocardia* spp. isolates were identified.
[c]A total of 15 environmental samples were collected during slaughter and examined for *Mycobacterium* spp.

proposed species of rapidly growing mycobacteria firstly isolated from the bone marrow of a human patient (Hoefsloot et al., 2010).

Of the lymph nodes from the 22 slaughtered cattle showing growth of *Mycobacterium* spp., 55% did not show macroscopic pathological lesions (Table 2). Amongst the lymph nodes showing macroscopic changes (n = 10), discoloration predominated (observed seven times), followed by bleeding and induration. *M. avium* subsp. *hominissuis* and *M. kansasii*, the predominant species of mycobacteria identified in this study, were found in lymph nodes with and without macroscopic changes. Histological examination of a subset of the mycobacteriologically analysed lymph nodes (n = 15) showed reactive hyperplasia (moderate to severe; n = 10; Figure 1, panel a), multifocal anthracosis (mild to moderate; n = 7; Figure 1, panel b), eosinophilic lymphadenitis (mild to moderate; n = 3), sinus histiocytosis (severe; n = 1) and plasmacellular hyperplasia (moderate; n = 1). Prussian blue stain was used to detect the presence of iron resulted negative, excluding haemosiderosis.

Amongst the 22 cattle from which *Mycobacterium* spp. were isolated from the lymph nodes, three (14%) of the corresponding faecal samples showed typical growth of *Mycobacterium* spp. and detection of acid-fast bacilli by Ziehl–Neelsen staining in the mycobacterial cultures. In addition, of the examined faecal samples, two tested positive for *Nocardia* spp. and one for *Rhodococcus equi*. The three mycobacterial strains isolated from faecal samples were identified as *M. hassiacum*, *M. phlei* and *M. vaccae*.

TABLE 2 Macroscopic and histological findings in bovine lymph nodes of slaughtered Swiss cattle positive for *Mycobacterium* spp

Examination of lymph nodes			
No.	Macroscopic findings[a]	Histological findings[b]	Species of *Mycobacteria* identified
8	–	+	*Mycobacterium avium* subsp. *hominissuis* (4) *Mycobacterium kansasii* (2) *Mycobacterium persicum* (2)
7	+	+	*Mycobacterium avium* subsp. *hominissuis* (4) *Mycobacterium kansasii* (2) *"Mycobacterium lymphaticum"* (1)
4	–	nd	*Mycobacterium avium* subsp. *hominissuis* (3) *Mycobacterium europaeum* (1)
3	+	nd	*Mycobacterium avium* subsp. *hominissuis* (3)

+, macroscopic or histological finding present; – no macroscopic or histological findings; nd, not done.

[a]Macroscopic findings comprised discoloration (predominantly), bleeding and induration.

[b]Histological findings comprised reactive hyperplasia (predominantly, moderate to severe), multifocal anthracosis (mild to moderate), eosinophilic lymphadenitis (mild to moderate), sinus histiocytosis (severe) and plasmacellular hyperplasia (moderate).

3.2 | Environmental samples

Of the 15 environmental samples collected during the slaughtering process, nine (60%) showed growth of *Mycobacteria* spp. in cultures. These nine samples originated from gloves (n = 3), the slaughter belt (n = 2), the wall (n = 2), a knife and a hook. The nine mycobacterial isolates were identified as *M. paragordonae* (n = 6), *M. peregrinum* (n = 2; from slaughter belt and wall) and *M. nebraskense* (n = 1, from the knife; Table 1).

3.3 | *Mycobacterium avium* complex characterization

For species identification and further characterization of MAC members, the complete hsp65-encoding gene was sequenced (and not only the common 441-bp portion). All 14 isolates were identified as *M. avium* subsp. *hominissuis*.

3.4 | *Mycobacterium kansasii* complex characterization

Phenotypically, the six *M. kansasii/persicum* isolates were not differentiable, showing characteristic slow growth with detection of colonies after approximatively 10 days of incubation at 37°C. All six isolates were classified as Runyon group I, producing a yellow to orange pigment after two hours of light exposure (photochromogenity). The 16S rRNA sequences of the six isolates presented three different sequevars (one single-point mutation). In the hypervariable fragment of the *hsp65* gene, two distinct clusters were observed. Two isolates (ZH39 and ZH97) were closer to *M. gastri* ATCC 15754 (eight point mutations) than to *M. kansasii* ATCC 12478 (14 point mutations). The other four isolates were closer to *M. kansasii* ATCC 12478 (one point mutation) than to *M. gastri* ATCC 15754 (13 point mutations). The phylogenetic tree based on *hsp65* showed clustering of the ZH39/ZH97 isolates with the recently described *M. persicum* DSM 104278 (Shahraki et al., 2017) and of the other four isolates with *M. kansasii* ATCC 12478. *M. bovis* ATCC 19210 was chosen as outgroup (Figure 2).

4 | DISCUSSION

In veterinary medicine, with the exception of *M. avium* subsp. *paratuberculosis*, the presence of NTM is generally not specifically searched (Biet & Boschiroli, 2014). Thus, isolation of NTM is often a secondary finding of MTBC investigations. Therefore, the large majority of publications report isolation of different NTM species within the framework of bTB surveillance programs. In the present study, identification and molecular characterization of NTM species isolated from healthy Swiss cattle at slaughter are described. The predominant species of mycobacteria identified belonged to the MAC. *M. avium* is a slowly growing mycobacterium and comprises four subspecies, namely *M. avium* subsp. *avium* (*Maa*), *M. avium* subsp. *silvaticum* (*Mas*), *M. avium* subsp. *hominissuis* (*Mah*) and

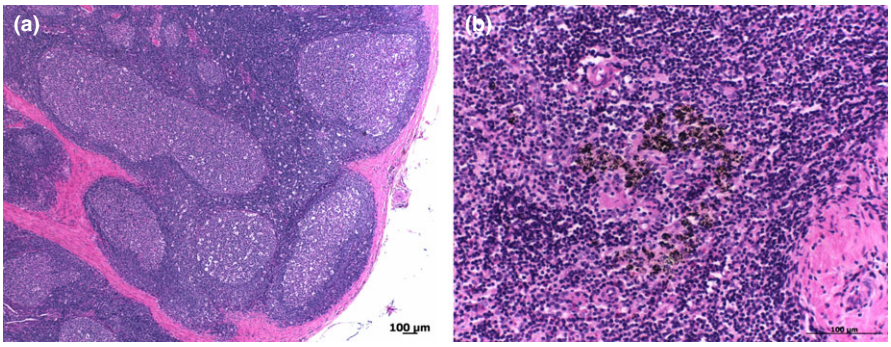

FIGURE 1 (a) Overview of an activated lymph node showing lymph follicles with tangible body macrophages interpreted as reactive follicular hyperplasia, haematoxylin and eosin (HE). (b) Lymph node showing black granular pigment in the cytoplasm of macrophages interpreted as moderate anthracosis (HE)

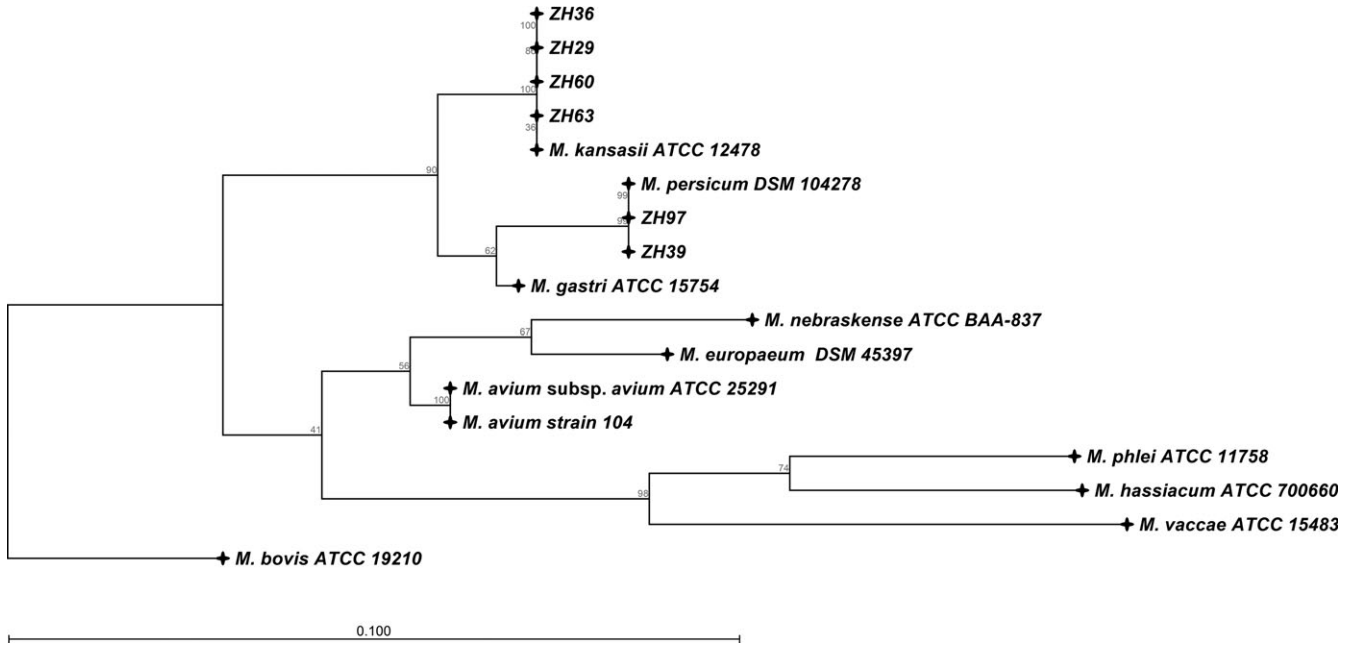

FIGURE 2 Phylogenetic tree based on *hsp65* gene sequences, assembled using the neighbour-joining method bootstrapped 1000 times. Bar, 0.1 substitutions per nucleotide position

M. avium subsp. *paratuberculosis* (*Map*). They are commonly differentiated by the presence or absence of specific insertion sequences, for example IS*900*, IS*901* and IS*1245*. Additionally, according to the current laboratory standard, the 3′ region of the *hsp65* gene can unambiguously distinguish between these subspecies (Turenne et al., 2006). Whilst *Maa* and *Mas* are the cause of avian tuberculosis and their molecular differentiation at subspecies level is under discussion, *Mah* has a clearly broader host range. Assuming that *Maa* and *Map* are obligate pathogens of birds and ruminants, respectively, and that these strains are rarely found in other hosts, a clear differentiation between environmental and host-specific members of the MAC is necessary. Nowadays, *Mah* is presumed a strict environmental (water, soil, dust and straw) mycobacterium and has been frequently isolated from different animals species, including pigs and cattle (Biet & Boschiroli, 2014; Mobius et al., 2006). Although infections with *Mah* are merely considered opportunistic, recent reports of transmission to an infection of immunocompetent and immunocompromised humans are increasing (Ichikawa et al., 2009; Leao et al., 2014; Maekura et al., 2005; Mijs et al., 2002; Mobius et al., 2006; Muwonge et al., 2014; Vluggen et al., 2016). The correct identification and characterization of MAC members is therefore crucial and may serve in defining subsets of epidemiological and clinical implications (Turenne et al., 2006). As the 108 slaughtered cattle of the present study were retained healthy and suitable for human consumption, the *Mah* infection rate of 13% in the left bronchial and caudal mediastinal lymph nodes is noteworthy and indicates a remarkable rate of subclinically infected cattle.

Of the NTM isolated from bovine lymph nodes in the present study, *Mah*, *M. kansasii* and *M. persicum* are known to have the potential to interfere with bTB diagnostics and, in some cases, to cause false-positive reactions leading to considerable economic losses (Vordermeier et al., 2007; Waters et al., 2006). In fact, the presence of the ESAT-6 and CFP-10 encoding genes in all cultured *M. kansasii* and *M. persicum* isolates (data not shown) demonstrates that infection by these strains may evoke sensitization and immune response in cattle. This might confound the interpretation of bTB tests, including the modern IFN-γ assay. Hence, the relatively high rate of infection by *M. kansasii* and *M. persicum* in this study could

be one explanation for the low specificity of the IFN-γ assay (Pucken et al., 2017).

Three isolates (ZH36, ZH60 and ZH63) belonged to *M. kansasii* subtype 1, generally considered more pathogenic for humans than other subtypes (2–7) (Taillard et al., 2003). According to the literature, subtype 1 is the most frequent subtype isolated from humans and it is only rarely isolated from the environment. Switzerland is faced with NTM infections in humans too (Kuznetcova, Sauty, & Herbort, 2012; Latshang, Lo Cascio, & Russi, 2011; Taillard et al., 2003); to the authors' knowledge, however, the number of infections is not considered to be higher than in neighbouring countries. The present findings in cattle show that *M. kansasii* subtype 1 does not exclusively infect humans. Moreover, this is the first report of a *M. persicum* infection in animals. With regard to the recently described pathogenicity of *M. persicum* in humans, further investigations are required to elucidate the route of transmission. Various NTM are ubiquitous in the environment and confirmed cases of direct transmission are rare. However, infected animals may shed the pathogen through their secretions, contaminating water and soil and representing a threat for other animals and humans. To evaluate the presence of NTM in the faeces of infected cattle, their intestinal flora has been submitted for mycobacteriological analysis. The three mycobacterial strains isolated from the positive faecal samples were identified as *M. hassiacum*, *M. phlei* and *M. vaccae*. In the three corresponding animals, *M. avium* subsp. *hominissuis* was cultured from the lymph nodes tested. The small number of animals analysed (n = 22) hampers a final conclusion but demonstrates the presence of viable NTM in bovine faeces. The failure to identify from stool potentially harmful NTM that were present in the lymph nodes may indicate that these are not shed into the environment by infected cattle through the faecal route. The three detected mycobacterial species from faecal samples are to date retained environmental saprophytes and were already isolated from bovine faeces (Glanemann, Hoelzle, & Wittenbrink, 2002). So far, their clinical relevance has been argued, particularly in immunocompromised individuals where occasional infections have been described (Khatter, Singh, Arora, Rana, & Seth, 2008).

To investigate possible cross-contaminations at the abattoir, 15 environmental swabs collected during the slaughter process were submitted for mycobacterial culture. Mycobacterial species identified amongst isolates from the slaughterhouse environment thereby clearly differed from those from cattle (lymph nodes and faecal samples). The predominant mycobacterial species from the environment was *M. paragordonae* with six isolates. *M. paragordonae* is a slow-growing mycobacterium closely related to *M. gordonae*, which is typically regarded as a contaminant and commonly isolated from soil and water (Griffith et al., 2007; Thomas, Herrera-Rimann, Blanc, & Greub, 2006).

In conclusion, a remarkable number of mycobacterial infections were observed amongst clinically healthy Swiss cattle slaughtered for human consumption. With the purpose of improving bTB and paratuberculosis surveillance programs, the presence of various NTM species should be taken into account. Moreover, although the zoonotic risk of NTM isolated from Swiss cattle remains unclear, it must be emphasized that human pathogenic strains have been identified and further epidemiological investigation including isolates from different sources are required.

ACKNOWLEDGEMENTS

The authors would like to thank Enrico Tortoli for providing *hsp65* gene sequences of *M. persicum* isolates and for constructive exchange of opinions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

G. Ghielmetti http://orcid.org/0000-0002-3936-9687

REFERENCES

Adékambi, T., Colson, P., & Drancourt, M. (2003). rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. *Journal of Clinical Microbiology*, *41*, 5699–5708. https://doi.org/10.1128/JCM.41.12.5699-5708.2003

Agdestein, A., Olsen, I., Jorgensen, A., Djonne, B., & Johansen, T. B. (2014). Novel insights into transmission routes of *Mycobacterium avium* in pigs and possible implications for human health. *Veterinary Research*, *45*, 46. https://doi.org/10.1186/1297-9716-45-46

Biet, F., & Boschiroli, M. L. (2014). Non-tuberculous mycobacterial infections of veterinary relevance. *Research in Veterinary Science*, *97* (Suppl), S69–S77. https://doi.org/10.1016/j.rvsc.2014.08.007

Biet, F., Boschiroli, M. L., Thorel, M. F., & Guilloteau, L. A. (2005). Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium*-intracellulare complex (MAC). *Veterinary Research*, *36*, 411–436. https://doi.org/10.1051/vetres:2005001

Falkinham, J. O. 3rd (1996). Epidemiology of infection by nontuberculous mycobacteria. *Clinical Microbiology Reviews*, *9*, 177–215.

Falkinham, J. O. 3rd (2009). Surrounded by mycobacteria: Nontuberculous mycobacteria in the human environment. *Journal of Applied Microbiology*, *107*, 356–367. https://doi.org/10.1111/j.1365-2672.2009.04161.x

Falkinham, J. O. 3rd (2015). Environmental sources of nontuberculous mycobacteria. *Clinics in Chest Medicine*, *36*, 35–41. https://doi.org/10.1016/j.ccm.2014.10.003

Gcebe, N., Rutten, V., Gey van Pittius, N. C., & Michel, A. (2013). Prevalence and distribution of non-tuberculous mycobacteria (NTM) in cattle, African buffaloes (Syncerus caffer) and their environments in South Africa. *Transboundary and Emerging Diseases*, *60*(Suppl 1), 74–84. https://doi.org/10.1111/tbed.12133

Ghielmetti, G., Scherrer, S., Friedel, U., Frei, D., Suter, D., Perler, L., & Wittenbrink, M. M. (2017). Epidemiological tracing of bovine tuberculosis in Switzerland, multilocus variable number of tandem repeat analysis of *Mycobacterium bovis* and *Mycobacterium caprae*. *PLoS ONE*, *12*, e0172474. https://doi.org/10.1371/journal.pone.0172474

Glanemann, B., Hoelzle, L. E., & Wittenbrink, M. M. (2002). Bacteriological investigations about paratuberculosis in dairy herds in Switzerland. *DTW. Deutsche Tierarztliche Wochenschrift*, *109*, 528–529.

Griffith, D. E., Aksamit, T., Brown-Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F., … Winthrop, K. (2007). An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American Journal of Respiratory and Critical Care Medicine*, *175*, 367–416. https://doi.org/10.1164/rccm.200604-571ST

Hoefsloot, W., vanIngen, J., vanSoolingen, D., Tortoli, E., Dekhuijzen, R., & Boeree, M. J. (2010). *Mycobacterium lymphaticum* sp. nov., a pathogenic rapidly growing species National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, Netherlands. Accession Number: HQ127228.1

Hope, J. C., Thom, M. L., Villarreal-Ramos, B., Vordermeier, H. M., Hewinson, R. G., & Howard, C. J. (2005). Exposure to *Mycobacterium avium* induces low-level protection from *Mycobacterium bovis* infection but compromises diagnosis of disease in cattle. *Clinical and Experimental Immunology*, *141*, 432–439. https://doi.org/10.1111/j.1365-2249.2005.02882.x

Ichikawa, K., Yagi, T., Moriyama, M., Inagaki, T., Nakagawa, T., Uchiya, K., … Ogawa, K. (2009). Characterization of *Mycobacterium avium* clinical isolates in Japan using subspecies-specific insertion sequences, and identification of a new insertion sequence, ISMav6. *Journal of Medical Microbiology*, *58*, 945–950. https://doi.org/10.1099/jmm.0.008623-0

Katale, B. Z., Mbugi, E. V., Botha, L., Keyyu, J. D., Kendall, S., Dockrell, H. M., … Matee, M. I. (2014). Species diversity of non-tuberculous mycobacteria isolated from humans, livestock and wildlife in the Serengeti ecosystem. *BMC Infectious Diseases*, *14*, 616. https://doi.org/10.1186/s12879-014-0616-y

Khatter, S., Singh, U. B., Arora, J., Rana, T., & Seth, P. (2008). Mycobacterial infections in human immuno-deficiency virus seropositive patients: Role of non-tuberculous mycobacteria. *The Indian Journal of Tuberculosis*, *55*, 28–33.

Kim, B. R., Kim, J. M., Kim, B. J., Jang, Y., Ryoo, S., Kook, Y. H., & Kim, B. J. (2014). Identification of nontuberculous mycobacteria isolated from Hanwoo (Bos taurus coreanae) in South Korea by sequencing analysis targeting hsp65, rpoB and 16S rRNA genes. *Veterinary Microbiology*, *173*, 385–389. https://doi.org/10.1016/j.vetmic.2014.07.019

Kirschner, P., & Böttger, E. C. (2000). Species identification of mycobacteria using rDNA sequencing. In T. Parish & N. G. Stoker (Eds.), *Methods in Molecular Biology* (pp. 349–361). Totowa, NJ, USA: Humana Press Inc.

Klanicova-Zalewska, B., & Slana, I. (2014). Presence and persistence of *Mycobacterium avium* and other nontuberculous mycobacteria in animal tissues and derived foods: A review. *Meat Science*, *98*, 835–841. https://doi.org/10.1016/j.meatsci.2014.08.001

Kuznetcova, T. I., Sauty, A., & Herbort, C. P. (2012). Uveitis with occult choroiditis due to *Mycobacterium kansasii*: Limitations of interferon-gamma release assay (IGRA) tests (case report and mini-review on ocular non-tuberculous mycobacteria and IGRA cross-reactivity). *International Ophthalmology*, *32*, 499–506. https://doi.org/10.1007/s10792-012-9588-3

Lara, G. H., Ribeiro, M. G., Leite, C. Q., Paes, A. C., Guazzelli, A., da Silva, A. V., … Listoni, F. J. (2011). Occurrence of *Mycobacterium* spp. and other pathogens in lymph nodes of slaughtered swine and wild boars (Sus scrofa). *Research in Veterinary Science*, *90*, 185–188. https://doi.org/10.1016/j.rvsc.2010.06.009

Latshang, T. D., Lo Cascio, C. M., & Russi, E. W. (2011). Nontuberculous mycobacterial infections of the lung. *Therapeutische Umschau.*, *68*, 402–406. https://doi.org/10.1024/0040-5930/a000184

Leao, C., Canto, A., Machado, D., Sanches, I. S., Couto, I., Viveiros, M., … Botelho, A. (2014). Relatedness of *Mycobacterium avium* subspecies hominissuis clinical isolates of human and porcine origins assessed by MLVA. *Veterinary Microbiology*, *173*, 92–100. https://doi.org/10.1016/j.vetmic.2014.06.027

Maekura, R., Okuda, Y., Hirotani, A., Kitada, S., Hiraga, T., Yoshimura, K., … Ito, M. (2005). Clinical and prognostic importance of serotyping *Mycobacterium avium*-Mycobacterium intracellulare complex isolates in human immunodeficiency virus-negative patients. *Journal of Clinical Microbiology*, *43*, 3150–3158. https://doi.org/10.1128/JCM.43.7.3150-3158.2005

Mijs, W., de Haas, P., Rossau, R., Van der Laan, T., Rigouts, L., Portaels, F., & van Soolingen, D. (2002). Molecular evidence to support a proposal to reserve the designation *Mycobacterium avium* subsp. avium for bird-type isolates and '*M. avium* subsp. hominissuis' for the human/porcine type of *M. avium*. *International Journal of Systematic and Evolutionary Microbiology*, *52*, 1505–1518. https://doi.org/10.1099/00207713-52-5-1505

Mobius, P., Lentzsch, P., Moser, I., Naumann, L., Martin, G., & Kohler, H. (2006). Comparative macrorestriction and RFLP analysis of *Mycobacterium avium* subsp. avium and *Mycobacterium avium* subsp. hominissuis isolates from man, pig, and cattle. *Veterinary Microbiology*, *117*, 284–291. https://doi.org/10.1016/j.vetmic.2006.05.005

Muwonge, A., Oloya, J., Kankya, C., Nielsen, S., Godfroid, J., Skjerve, E., … Johansen, T. B. (2014). Molecular characterization of *Mycobacterium avium* subspecies hominissuis isolated from humans, cattle and pigs in the Uganda cattle corridor using VNTR analysis. *Infection, Genetics and Evolution*, *21*, 184–191. https://doi.org/10.1016/j.meegid.2013.11.012

Prevots, D. R., & Marras, T. K. (2015). Epidemiology of human pulmonary infection with nontuberculous mycobacteria: A review. *Clinics in Chest Medicine*, *36*, 13–34. https://doi.org/10.1016/j.ccm.2014.10.002

Primm, T. P., Lucero, C. A., & Falkinham, J. O. 3rd (2004). Health impacts of environmental mycobacteria. *Clinical Microbiology Reviews*, *17*, 98–106. https://doi.org/10.1128/CMR.17.1.98-106.2004

Procop, G. W. (2017). HIV and mycobacteria. *Seminars in Diagnostic Pathology*, *34*, 332–339. https://doi.org/10.1053/j.semdp.2017.04.006

Pucken, V. B., Knubben-Schweizer, G., Dopfer, D., Groll, A., Hafner-Marx, A., Hormansdorfer, S., … Hartnack, S. (2017). Evaluating diagnostic tests for bovine tuberculosis in the southern part of Germany: A latent class analysis. *PLoS ONE*, *12*, e0179847. https://doi.org/10.1371/journal.pone.0179847

Ronai, Z., Eszterbauer, E., Csivincsik, A., Guti, C. F., Dencso, L., Janosi, S., & Dan, A. (2016). Detection of wide genetic diversity and several novel strains among non-avium nontuberculous mycobacteria isolated from farmed and wild animals in Hungary. *Journal of Applied Microbiology*, *121*, 41–54. https://doi.org/10.1111/jam.13152

de la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science*, *81*, 190–210. https://doi.org/10.1016/j.rvsc.2005.11.005

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*, 406–425.

Schiller, I., Oesch, B., Vordermeier, H. M., Palmer, M. V., Harris, B. N., Orloski, K. A., … Waters, W. R. (2010). Bovine tuberculosis: A review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transboundary and Emerging Diseases*, *57*, 205–220. https://doi.org/10.1111/j.1865-1682.2010.01148.x

Shahraki, A. H., Trovato, A., Mirsaeidi, M., Borroni, E., Heidarieh, P., Hashemzadeh, M., … Tortoli, E. (2017). *Mycobacterium persicum* sp. nov., a novel species closely related to *Mycobacterium kansasii* and *Mycobacterium gastri*. *International Journal of Systematic and Evolutionary Microbiology*, *67*, 1766–1770. https://doi.org/10.1099/ijsem.0.001862

Taillard, C., Greub, G., Weber, R., Pfyffer, G. E., Bodmer, T., Zimmerli, S., … Prod'hom, G. (2003). Clinical implications of *Mycobacterium kansasii* species heterogeneity: Swiss National Survey. *Journal of Clinical Microbiology*, *41*, 1240–1244. https://doi.org/10.1128/JCM.41.3.1240-1244.2003

Telenti, A., Marchesi, F., Balz, M., Bally, F., Bottger, E. C., & Bodmer, T. (1993). Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *Journal of Clinical Microbiology*, *31*, 175–178.

Thacker, T. C., Robbe-Austerman, S., Harris, B., Van Palmer, M., & Waters, W. R. (2013). Isolation of mycobacteria from clinical samples collected in the United States from 2004 to 2011. *BMC Veterinary Research*, *9*, 100. https://doi.org/10.1186/1746-6148-9-100

Thomas, V., Herrera-Rimann, K., Blanc, D. S., & Greub, G. (2006). Biodiversity of amoebae and amoeba-resisting bacteria in a hospital water network. *Applied and Environment Microbiology*, *72*, 2428–2438. https://doi.org/10.1128/AEM.72.4.2428-2438.2006

Tortoli, E. (2003). Impact of genotypic studies on mycobacterial taxonomy: The new mycobacteria of the 1990s. *Clinical Microbiology Reviews*, *16*, 319–354. https://doi.org/10.1128/CMR.16.2.319-354.2003

Turenne, C. Y., Semret, M., Cousins, D. V., Collins, D. M., & Behr, M. A. (2006). Sequencing of hsp65 distinguishes among subsets of the *Mycobacterium avium* complex. *Journal of Clinical Microbiology*, *44*, 433–440. https://doi.org/10.1128/JCM.44.2.433-440.2006

Vluggen, C., Soetaert, K., Duytschaever, L., Denoel, J., Fauville-Dufaux, M., Smeets, F., … Mathys, V. (2016). Genotyping and strain distribution of *Mycobacterium avium* subspecies hominissuis isolated from humans and pigs in Belgium, 2011-2013. *Eurosurveillance Weekly*, *21*, 30111. https://doi.org/10.2807/1560-7917.ES.2016.21.3.30111

Vordermeier, H. M., Brown, J., Cockle, P. J., Franken, W. P., Drijfhout, J. W., Arend, S. M., … Hewinson, R. G. (2007). Assessment of cross-reactivity between *Mycobacterium bovis* and *M. kansasii* ESAT-6 and CFP-10 at the T-cell epitope level. *Clinical and Vaccine Immunology*, *14*, 1203–1209. https://doi.org/10.1128/CVI.00116-07

Waters, W. R., Palmer, M. V., Thacker, T. C., Payeur, J. B., Harris, N. B., Minion, F. C., … Lyashchenko, K. P. (2006). Immune responses to defined antigens of *Mycobacterium bovis* in cattle experimentally infected with *Mycobacterium kansasii*. *Clinical and Vaccine Immunology*, *13*, 611–619. https://doi.org/10.1128/CVI.00054-06

How to cite this article: Ghielmetti G, Friedel U, Scherrer S, et al. Non-tuberculous *Mycobacteria* isolated from lymph nodes and faecal samples of healthy slaughtered cattle and the abattoir environment. *Transbound Emerg Dis*. 2017;00: 1–8. https://doi.org/10.1111/tbed.12793